## Assessment Run B11 2011

# HER2 BRISH

### Material

The material circulated for the BRISH (Bright field In Situ Hybridization) HER-2 assessment run B11 comprised one normal breast tissue and five breast ductal carcinomas showing HER-2 gene/chromosome 17 (HER-2/chr17) ratios as follows:


| | HER-2 IHC* | Dual - SISH** | FISH*** |
|---|---|---|---|
| | IHC score | HER-2/chr17 ratio | HER-2/chr17 ratio |
| 1. Normal breast tissue | 0 | 1.1 - 1.2 | 1.1 - 1.3 |
| 2. Breast ductal carcinoma | 3+ | 3.5 - 4.0 | 4.3 - 5.5 |
| 3. Breast ductal carcinoma | 1+ | 1.2 - 1.4 | 1.3 - 1.5 |
| 4. Breast ductal carcinoma | 2+ | 1.8 - 2.1 | 2.1 - 2.4 |
| 5. Breast ductal carcinoma | 2+ | 1.9 - 2.1 | 2.0 - 2.4 |
| 6. Breast ductal carcinoma* | 2+ | 1.6 - 2.0 | 1.7 - 2.2 |

*PATHWAY®, Ventana (data from one reference lab.)
**INFORM™ HER-2 Dual SISH kit, Ventana (range of data from two reference labs.)
***HER2 FISH pharmDX™ Kit, Dako (range of data from two reference labs.).
All tissues were fixed for 24 - 48 h. in 10 % neutral buffered formalin (NBF).

Criteria for assessing a BRISH HER-2 analysis as optimal included:

- Staining of the normal breast tissue and the ductal carcinoma no. 3 corresponding a non-amplified status.
- Staining of the breast ductal carcinoma no. 2 corresponding a (highly) amplified status.
- Staining of the breast ductal carcinoma no. 4 & 5 corresponding to an equivocal or low amplified status.
- Staining with preserved morphological details and a minimal background reaction.

*The breast ductal carcinoma no. 6 was only evaluated regarding the ability to demonstrate the HER-2 signals in both the normal cells and neoplastic cells, whereas the participating laboratories were not evaluated regarding the interpretation, as the tumour showed a range from non-amplified to low amplified in the reference laboratories.

A staining was assessed as good, if the above mentioned criteria were fulfilled, but the interpretation was slightly compromised e.g., due to a weak or excessive counterstaining, excessive retrieval or similar.

A staining was assessed as borderline if one of the tissue cores could not be properly evaluated due to a too weak signal or a low signal-to-noise ratio.

A staining was assessed as poor if two or more of the tissue cores could not be properly evaluated.

### Results

65 laboratories participated in this assessment. 41 (63 %) achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Systems and assessment marks for BRISH HER-2, run B11**

| **Two colour HER-2 systems** | **N** | **Vendor** | **Optimal** | **Good** | **Borderl.** | **Poor** | **Suff.[1]** |
|---|---|---|---|---|---|---|---|
| INFORM™ HER-2 Dual SISH **780-4332+780-4331, 800-4422** | 38 | Ventana | 14 | 5 | 16 | 3 | 50 % |
| DuoCISH™ **SK108 & SK109** | 10 | Dako | 4 | 3 | 0 | 3 | 70 % |
| ZytoDot®2C **C-3022-40** | 2 | ZytoVision | 1 | 1 | 0 | 0 | - |
| **One colour HER-2** | | | | | | | |

| systems | | | | | | | |
|---|---|---|---|---|---|---|---|
| INFORM™ HER-2 SISH **780-4332** | 8 | Ventana | 4 | 3 | 1 | 0 | 89 % |
| ZytoDot® **C-3003-40** | 4 | ZytoVision | 2 | 1 | 0 | 1 | - |
| SPOT-Light® **84-0150** | 2 | Invitrogen | 2 | 0 | 0 | 0 | - |
| "In-house" | 1 | | 1 | 0 | 0 | 0 | - |
| **Total** | 65 | | 28 | 13 | 17 | 7 | - |
| **Proportion** | | | 43 % | 20 % | 26 % | 11 % | 63 % |

1) Proportion of sufficient stains.

**Comments**

In this assessment an optimal demonstration and evaluation of the HER-2 gene amplification status in all the tissues included in the multi block could be obtained by all the different systems used by the laboratories.

In accordance with the previous runs for HER-2 BRISH, the INFORM™ Dual SISH system, Ventana and the DuoCISH™, Dako were the two most widely BRISH systems used.

All the included tissues were fixed in 10 % neutral buffered formalin for 24-48 hours according to the ASCO/CAP guidelines for breast tissue. Even though same fixation and tissue processing conditions were identical for the 6 included tissues it was observed, that the breast ductal carcinoma no. 3 and in particular no. 4 were more challenging than the other tissues regarding the protocol settings. In these two tumours the ability to demonstrate the HER-2 signals was highly influenced by the pre-treatment conditions as excessive retrieval typically impaired the morphology as the nuclei were almost totally digested with consequent loss of the BRISH signals. This pattern was seen for all systems used.

For the **INFORM™ Dual SISH system**, Ventana an optimal demonstration for HER-2 BRISH was in brief typically based upon HIER in Cell Conditioning 2 (CC2) for 28 min. at 86-90˚C and proteolysis in P3 for 8 - 12 min. at 37˚C. The HER-2 SISH probe was typically applied for 6 hours at 50-52˚C, while the chr17 probe was applied for 2 hours at 42 - 44˚C.

An insufficient result for the INFORM™ Dual SISH system, Ventana was seen in 50 % of the submitted protocols and was typically due to an excessive retrieval hampering the interpretation as the nuclei were almost totally digested complicating the identification and interpretation of the BRISH signals. This was typically seen by using CC2 for > 28 min. combined with proteolysis in P3 for 12 - 20 min. These protocol settings were applied by 10 laboratories. 5 laboratories used a protocol with optimal settings, but for unexplained reasons a complete false negative staining or a staining result with an excessive background staining e.g. due to silver precipitates was seen.

For the **DuoCISH™ system**, Dako, the main protocol settings giving an optimal result were based on HIER for 10 min in the pre-treatment buffer at 95 - 97°C and proteolysis for 2 min. in Pepsin at 37°C (both reagents included in the FISH pharmDX kit K5331, Dako or CISH kit SK108/109). The HER-2 and the chr17 probe (SK109/K5331, Dako) was applied for 14 – 20 hours at 45˚C and visualized by the DuoCISH™ kit SK108/109, Dako. For the Dako DuoCISH™ system the prevalent feature of an insufficient result typically was a generally too weak or completely negative reaction of the HER-2 signals in both the neoplastic cells and in the normal stromal cells and most likely related to insufficient proteolysis in Pepsin – too short time and/or reduced enzymatic capacity of the applied Pepsin. Pepsin is a relative fragile enzyme and rapidly deteriorates if stored at room temperature. Pepsin should always be stored at 2 - 6°C and kept on ice when taken out of the refrigerator to secure optimal performance.

In concordance to previous runs the insufficient results were mainly related to the demonstration of the HER-2 signals, whereas the chr17 signals were distinctively demonstrated. This observation might be related to a too low sensitivity of the reagents used for the immunohistochemical demonstration of the HER-2 genes. In this context it has to be stressed that it is of utmost importance that the Red chromogene

used for the visualization of the HER-2 genes in the DuoCISH™ system is prepared immediately before use.

The newly lauched **ZytoDot®2C CISH system**, ZytoVision gave an optimal result by using proteolysis in Pepsin for 9 min at room temp, HIER in EDTA for 15 min. at 95˚C, hybridization at 37˚C for 14-16 hours and visualized by the ZytoVision detection kit C3022-40. The protocols were applied according to the recommendations given in the package insert from ZytoVision.

The laboratories were requested to send in their own interpretation on the stained sections, which was completed by 37 out of the 41 laboratories obtaining a sufficient mark (optimal or good). 13 out of these (35 %) interpreted and classified the tumours in concordance to the HER-2/chr17 statuses generated in the reference laboratories. In this context it has to be mentioned that the tumour no. 6 was excluded due to non-conclusive data from the reference laboratories.
The discrepancies between the interpretations were mainly related to the breast ductal carcinomas no. 3 and 5. 8 laboratories interpreted the breast carcinoma no. 3 as either equivocal (n=4) or amplified (n=4). This tumour was classified as 1+ by IHC for HER-2 and showed a HER-2/chr17 ratio of 1.3 - 1.5 by FISH. 11 laboratories classified the breast carcinoma no. 5 as non-amplified, whereas this tumour was classified as 2+ by IHC and low level HER-2 gene amplification, 2.0 - 2.4 by the reference laboratories.
The 3 breast ductal carcinomas no. 4 - 6 were all very challenging regarding the interpretation as all 3 either showed an equivocal or low level of amplification, which most likely explains the low proportion of concordant interpretations between the laboratories and the reference data / NordiQC. A consensus rate of 97 % (36 out of 37 laboratories) between the participants and NordiQC was seen regarding the interpretation in the normal breast tissue no. 1 and the breast ductal carcinoma no. 2 with a high level of amplification.

This was the 5’ assessment of HER-2 BRISH in NordiQC and as seen in table 2, a lower pass rate and proportion of sufficient results was seen in this run compared to the previous assessments.

Table 2. **Proportion of sufficient results for HER-2 BRISH in the NordiQC runs performed**

| | Run C1 2009 | Run C2 2009 | Run B9 2010 | Run B10 2010 | Run B11 2011 |
|---|---|---|---|---|---|
| Participants, n= | 17 | 34 | 53 | 57 | 65 |
| Sufficient results | 88 % | 68 % | 72 % | 72 % | 63 % |

## Conclusion

In this assessment an optimal demonstration of HER-2 BRISH could be obtained by the commercially available two-colour HER-2 systems INFORM™ HER-2 Dual SISH, Ventana, DuoCISH™,Dako and ZytoDot®2c, ZytoVision. Also the single-colour HER-2 systems, INFORM™ HER-2 Dual SISH, Ventana, ZytoDot ®, ZytoVision and SPOT-Light®, Invitrogen could be used to obtain an optimal demonstration. For an optimal performance the retrieval settings – HIER + proteolysis - must be carefully balanced between high sensitivity and preserved morphology. Attention must also be addressed to the interpretation as only 37 % of the laboratories obtained a sufficient result and gave an interpretation in concordance to the reference data.

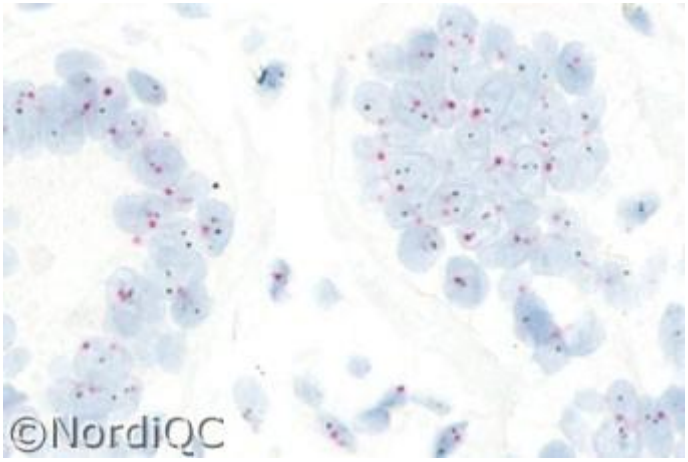


Fig. 1a
Optimal staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the normal breast tissue no. 1 without gene amplification: HER-2/chr17 ratio 1.1 - 1.3*.
The HER-2 gene is stained black, while chr17 is stained red.

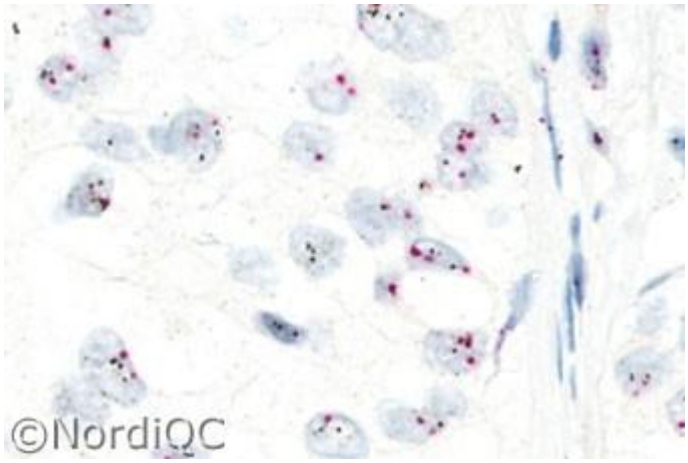


Fig. 1b
Optimal staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 3 without gene amplification: HER-2/chr17 ratio 1.3 – 1.5*.
The HER-2 gene is stained black, while chr17 is stained red.

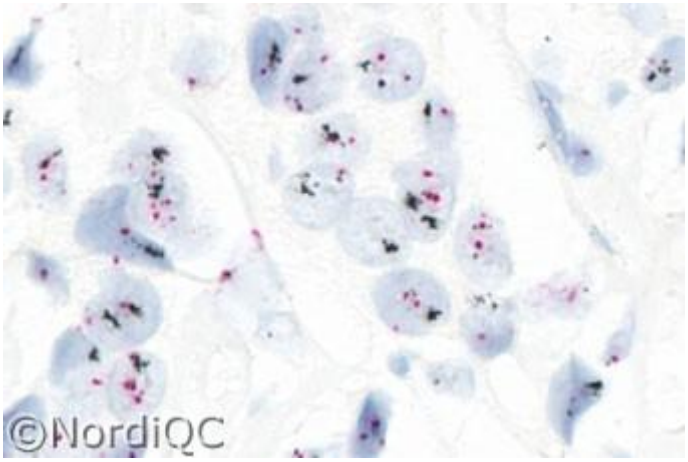


Fig. 2a
Optimal staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 2 with a high level of gene amplification: HER-2/chr17 ratio 4.3 – 5.5*.
The HER-2 gene is stained black and located in clusters, while chr17 is stained red.

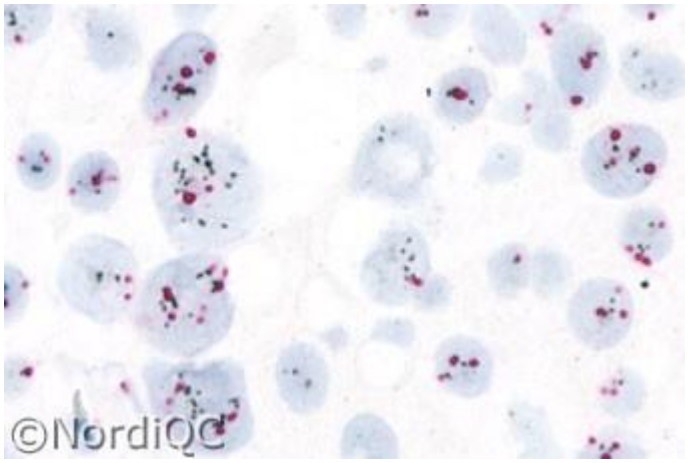


Fig. 2b
Optimal staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 5 with a low level of HER-2 gene amplification: HER-2/chr17 ratio 2.0 – 2.4*.
The HER-2 gene is stained black (increased number & small clusters), while chr17 is stained red (increased number).

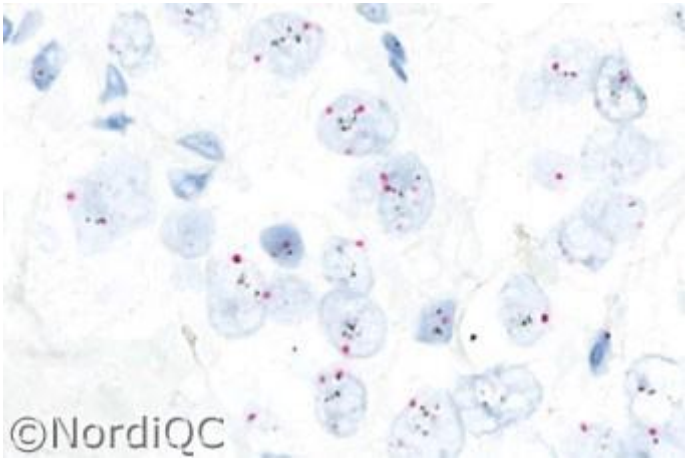


Fig. 3a
Optimal staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification: HER-2/chr17 ratio 2.1 – 2.4*.
The HER-2 gene is stained black, while chr17 is stained red.

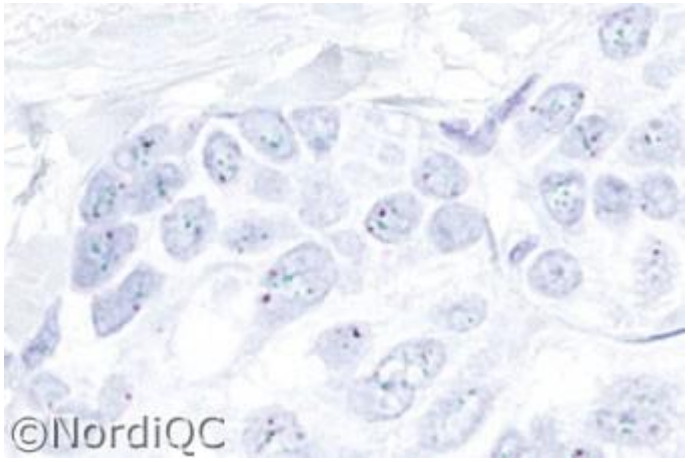


Fig. 3b
Optimal staining for the HER-2 gene status using the ZytoDot®2C CISH kit, ZytoVision of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification: HER-2/chr17 ratio 2.1 – 2.4*.
The HER-2 gene is stained green, while chr17 is stained red.

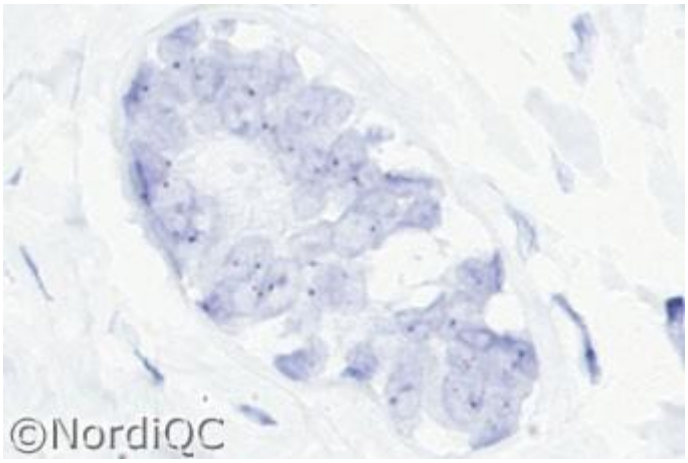


Fig. 4a
Insufficient staining for the HER-2 gene status using the DuoCISH™, Dako of the normal breast tissue no. 1 without gene amplification. No HER-2 signals can be identified, and only the chr17 signals can be seen. This aberrant reaction may be caused by e.g., too short HIER, too short proteolysis with Pepsin (or Pepsin with reduced activity, due to storage at room temp.) or less successful IHC staining for the HER2 genes.

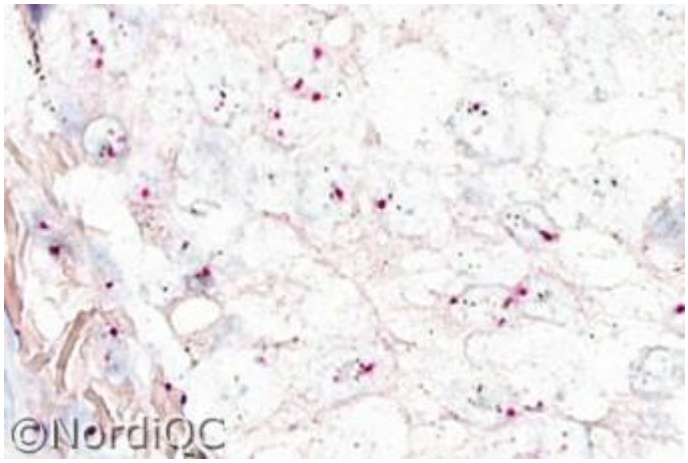


Fig. 4b
Insufficient staining for the HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 3 without HER-2 gene amplification. Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.

* Reference: HER-2 FISH pharmDX™ Kit, Dako (range of ratio data from two reference laboratories).

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